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Morphological cladistic analysis as a model for character evaluation in primitive living chitons (Polyplacophora, Lepidopleurina)*

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Abstract: Chitons are often referred to as "living fossils" in part because they are proposed as one of the earliest-diverging groups of living molluscs, but also because the gross morphology of the polyplacophoran shell has been conserved for hundreds of millions of years. As such, the analysis of evolution and radiation within polyplacophorans is of considerable interest not only for resolving the shape of pan-molluscan phylogeny but also as model organisms for the study of character evolution. This study presents a new, rigorous cladistic analysis of the morphological characters used in taxonomic descriptions for chitons in the living suborder Lepidopleurina Thiele, 1910 (the earliest-derived living group of chitons). Shell-based characters alone entirely fail to recover any recognized subdivisions within the group, which may raise serious questions about the application of fossil data (from isolated shell valves). New analysis including characters from girdle armature and gill arrangements recovers some genera within the group but also points to the lack of monophyly within the main genus *Leptochiton* Gray, 1847. Additional characters from molecular data and soft anatomy, used in combination, are clearly needed to resolve questions of chiton relationships. However, the data sets currently available already provide interesting insights into the analytical power of traditional morphology as well as some knowledge about the early evolution and radiation of this group.

Key words: morphology, molluscan evolution, cladistics, *Leptochiton*

Phylogenetic studies have shown that chitons (Polyplacophora) retain many features that are plesiomorphic within molluscs and indeed appear to have close morphological similarity with the hypothesized common ancestor of the Mollusca (*e.g.*, Haszprunar 1996, Sigwart and Sutton 2007; Fig. 1A). Nevertheless, given the radical disparity encompassed by molluscan morphology, understanding the patterns of evolution within the Polyplacophora is of particular importance to undertand how a *bauplan* that has remained so conserved over hundreds of millions of years in the direct living descendants of the ancestral chiton may have also given rise to forms as different as bivalves and cephalopods. The living chitons are contained in the subclass Neoloricata, which has a fossil record extending from the Carboniferous (ca. 350 Mya) to Recent. However, in spite of this deep fossil record, the majority of fossil species are known only from isolated, disarticulated plates, which makes it very difficult to infer the morphology of the whole animal (Cherns 2004, Vendrasco *et al.* 2004, Sigwart and Sutton 2007). The three major clades within the Neoloricata (Lepidopleurida, Chitonina, and Acanthochitonina) are separated stratigraphically in the fossil record as well as morphologically, which has resulted in a broad acceptance of their monophyly and phylogenetic separation at the ordinal level (Buckland-Nicks 1995, Okusu *et al.* 2003). The suborder Lepidopleurina is uncontroversially accepted to be the earliest-derived group of living chitons (Fig. 1B). In spite of this broadly accepted separation, to date there is limited understanding of the relationships between taxa within the major clades of chitons and not a robust testing of the monophyly of these groups across the broad range of taxa they each cover. Living members of the order Lepidopleurida (suborder Lepidopleurina) include nine genera comprising five families (*sensu* Sirenko 2006); but the genus *Leptochiton* Gray, 1847 contains about 80 of the 120 valid extant species in the order. Complex shell and girdle features that are used to define non-lepidopleuran chitons are often lacking in these species, which are small, plain, and superficially homogenous in their external morphology. However, species are described using the same conventional features across all chitons—shell sculpture, girdle armature microstructure, gills, and gross radular morphology. In addition, several morphological features, particularly from soft anatomy, have been proposed to assess the intra-clade relationships of chitons, particularly sperm acrosome and egg hull structures (Hodgson *et al.* 1988, Sirenko 1993, Buckland-Nicks 1995, 2006), relative positions of gonopores

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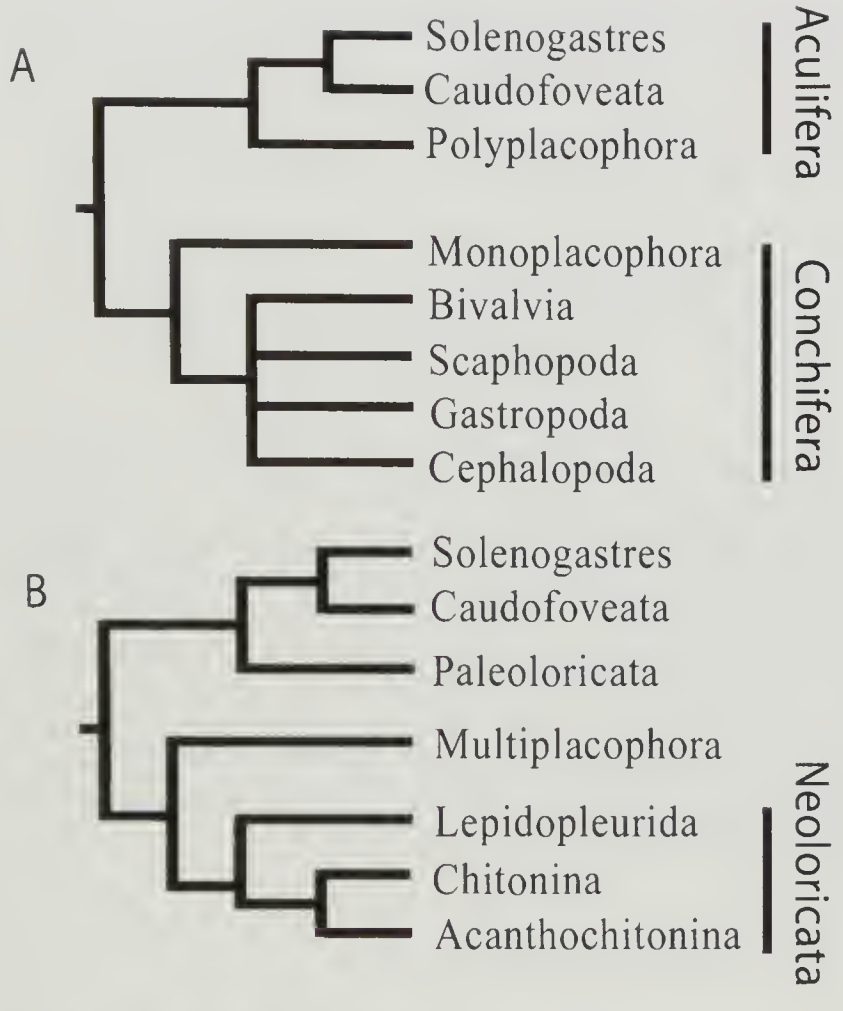

Figure 1. A, generalized topology of the aculiferan model of molluscan evolution, showing relationships between living classes of molluscs. B, generalized topology of major clades within the Aculifera (redrawn from Sigwart and Sutton 2007). All living chitons are classified in the subclass Neoloricata, in the two orders Chitonida (containing two suborders, Chitonina and Acanthochitonina) and Lepidopleurida (the focus of the present study).

and nephridiopores within the gill row (Sirenko 1993, Sigwart 2008b), and patterns of aesthete canals within the valves (Fernandez *et al.* 2007, Vendrasco *et al.* 2008).

The aim of this paper is to empirically assess the usefulness of the morphological features that are currently used to define species, by testing their ability to build phylogenetic hypotheses within and between the taxa that comprise this clade. This is accomplished here by using a subset of exemplar lepidopleuran taxa. The hypothesis tested is that if the characters used to diagnose and to describe species were defined expressly to separate species, genera, and other taxonomic subsets, then the expected results of a quantitative phylogenetic analysis of those characters would be a tree replicating the established Linnaean hierarchy. In fact, many non-diagnostic characters are regularly described for chitons, and the accepted diagnoses of genera in particular are fraught with inconsistency. Consequently, this study also examines the information content of morphological characters.

MATERIALS AND METHODS

Characters and taxa

This study draws on morphological examination by the author of preserved specimen material, particularly from the collections of the Museum national d'Histoire naturelle (MNHN, Paris); Zoologische Staatssammlung München (ZSM, Münich), Naturhistorisches Museum in Wien (NHMW, Vienna); and the National Museum of Ireland, Natural History Division (NMINH, Dublin). Sixty-nine morphological characters were described based on features used in every modern species description, derived from a standard implicitly set by the work of Kaas and Van Belle (1985). Character states are defined based on features identified in the literature and from the author's personal observation of specimen morphology. Codings for the present morphological matrix are based on original anatomical observation by the author. In principle, these character states are defined based on variability that is morphologically constant for all individuals within a species but may vary, encompassing the different states defined, in different species. The characters include 32 shell features, 27 girdle features, two aspects of gross body shape, two radula characters, and six characters of gill arrangement (Table 1). Part of this data set was based on the matrix of shell features published by Sigwart *et al.* (2007), incorporating new refinements to the characters used in that analysis. Characters were described with two (binary), three, or four potential character states. All characters were considered to be unordered (that is, evolutionary change could hypothetically transform freely between any of the described states) and without polarity (no ancestral state is assumed). All characters were equally weighted for the analysis. The complete character matrix is presented in Table 2. The characters were coded for 39 ingroup taxa selected to cover the morphological diversity of the living Lepidopleurina. Two species of *Callochiton* and the monotypic *Choriplax grayi* (H. Adams and Angas, 1864) were coded as outgroup taxa. The genus *Callochiton* resolved as sister to the family Leptochitonidae in a molecular phylogenetic analysis (Okusu *et al.* 2003). This is a controversial result which has not been supported by other studies (Buckland-Nicks 2006, Sirenko 2006, B. Lieb, pers. comm.). However, as there is so little information about the internal topology of any of the major polyplacophoran clades, this is the best evidence for choosing a most proximal outgroup from living taxa. *Choriplax* has traditionally been classified within the Lepidopleurina but has recently been considered by Sirenko (2006) to be an advanced form with secondarily derived features in common with lepidopleurans.

Phylogenetic analysis, consensus, and support

The complete matrix was initially subjected to a permutation tail probability test (PTP) to assess data quality, using

Table 1. Characters formulated from external morphology and used to code chiton taxa in the phylogenetic analysis. CI (confidence index) from main analysis of complete matrix.

	Character description	CI
1	Ratio: apophyses outside diameter / valve width: ≤0.8 (0); >0.8 (1).	0.125
2	Ratio: combined diameter of apophyses / valve width: ≤0.4 (0); >0.4 (1).	0.125
3	Thickened on terminal margins: no (0); yes (1).	0.250
4	General character of arch (intermediate plates): straight sides (0); rounded (1); concave (2).	0.333
5	Dorsal elevation (height / width) of intermediate plates: ≤0.4 (0); >0.4 (1).	0.200
6	Valves beaked: no (0); yes (1).	0.111
7	Lateral area elevated on intermediate plates: no (0); yes (1).	0.091
8	Intermediate plates with distinct diagonal separating lateral areas: no (0); yes (1).	0.077
9	Apophyses' jugal margin: straight (0); concave (1).	0.091
10	Head valve shape: semicircular (0); shape < semicircle (1); shape > semicircle (2).	0.222
11	Tail valve shape: semicircular (0); shape < semicircle (1); shape > semicircle (2).	0.286
12	Mucro prominent: no (0); yes (1).	0.100
13	Mucro position: posterior (0); median (1); anterior (2).	0.095
14	Post-mucronal slope: straight (0); concave (1); convex (2).	0.125
15	Articulamentum character: weak, transparent (0); moderate, opaque at least in major central areas (1); strong, forming thickened calluses and/or extending into insertions (2).	0.182
16	Insertion plates present: no (0); yes (1).	0.333
17	Tail valve apophyses shape same as on intermediate valves: yes (0); no, difference(s) (1).	0.077
18	Intermediate valve shape: trapezoidal (0); rectangular (1); ovate or circular (2).	0.400
19	Intermediate valves: anterior margin: straight (0); concave (1); convex (2).	0.167
20	Intermediate valves: posterior margin: straight (0); convex (1); concave around apex (2).	0.182
21	Tegmentum—general sculpture: smooth (0); granulose (1); pustulose or joined (2).	0.250
22	Tegmentum—gradation of sculpture: regular (0); larger toward margin (1); faded posteriorly (2).	0.333
23	Tegmentum—dominant granule shape: no granules (0); roundish (1); square or irregular (2).	0.250
24	Central areas of intermediate valves—distinct jugal sculpture: not distinct from pleural area (0); longitudinally granulate in jugal area; pleural areas coarser (1); jugal sculpture in quincunx, grading to radiating longitudinal or diagonal series (2).	0.167
25	Intermediate valves—sculpture with longitudinal rows: no pattern (0); longitudinal rows (1); quincunx or diagonal series (2); irregular, wavy, or zigzag (3).	0.214
26	Intermediate valves—sculpture interstices: close / narrow or sandy (0); evenly distributed, series of granules may be coalescing / beading (1); widespread or punctured (2).	0.118
27	Areas of intermediate plates with corresponding sculpture as terminal plates: no (0); yes (1).	0.200
28	Ratio: apophyses inside separation / valve width: ≤0.4 (0); >0.4 (1).	0.100
29	Thick periostracum forming pustules: absent (0); present (1).	1.000
30	Valve carinated or keeled: no (0); semi-carinated or keeled at posterior (1); yes (2).	0.133
31	Lateral area sculpture in radiating rows: no pattern or quincunx (0); strong radiating rows (1); central sculpture continuing in longitudinal rows (2); wavy or zigzag (3).	0.300
32	Tail valve more than twice the length of intermediate valves: no (0); yes (1).	0.500
33	Dorsal girdle scales—length / width ratio of typical scale (total length / width at base): absent (0); square (1); length ≥1.5 × width (2); width ≥1.5 × length (3).	0.200
34	Dorsal girdle scales: absent (0); straight (1); distally curved (2).	0.333
35	Dorsal girdle scales or spicules—generalized density: "dense" (1); unremarkable (2); "sparse" (3).	0.133
36	Dorsal girdle scales—distribution: absent (0); regular (1); irregular (2).	0.200
37	Dorsal girdle scales or spicules—lateral size gradient: no, equal at all points (0); smaller toward margin (1); larger toward margin (2).	0.167
38	Dorsal girdle scales—shape: absent (0); square top (1); round top (2); pointed (3).	0.250
39	Dorsal girdle scales—texture: absent (0); smooth to weakly ribbed or striated (1); distinct ribs, more than 10 (2).	0.273

Table 1. (Continued)

	Character description	CI
40	Ventral girdle scales—length relative to typical dorsal scale: absent (0); equal length (1); shorter, half or less length (2); longer, 1.5 times length or more (3).	0.250
41	Ventral girdle scales—width relative to typical dorsal scale: absent (0); equal width (1); narrower half or less length (2); wider 1.5 times length or more (3).	0.250
42	Ventral girdle scales—texture: absent (0); smooth to weakly ribbed or striated (1); distinct ribs, 10 or less (1); distinct ribs, more than 10 (2).	0.286
43	Ventral girdle scales or spicules: absent (0); straight (1); distally curved (2).	0.667
44	Ventral girdle scales or spicules—shape: absent (0); elongate oval, round top or pointed scales (1); rectangular scales (2); spiculose (3).	0.375
45	Ventral girdle scales or spicules—generalized density: "dense" (1); unremarkable (2); "sparse" (3).	0.167
46	Ventral girdle scales or spicules—distribution: absent (0); regular (1); irregular (2).	0.286
47	Ventral girdle scales or spicules—lateral size gradient: no, equal at all points (0); smaller toward foot (1); larger toward foot (2).	0.500
48	Dorsal spicules, including intersegmental armature—length relative to major dorsal scales: absent (0); spicules form major dorsal coverage, scales absent (1); dorsal or intersegmental spicules not substantially longer than major dorsal scales (2); dorsal or intersegmental spicules substantially longer, three times length of dorsal scales or longer (3).	0.429
49	Dorsal spicules, including intersegmental armature—shape: absent (0); straight (1); distally curved (2).	0.500
50	Dorsal spicules, including intersegmental armature—terminal shape: absent (0); sharp, pointed (1); blunt (2).	0.182
51	Intersegmental spicules—complex base: absent or simple (0); spicules in chitinous cupules (1); spicules in complex *rigschaftnadel* (2).	0.167
52	Dorsal spicules, including intersegmental armature—texture: absent (0); smooth, strongly tapered (1); striated or grooved (2); smooth, cylindrical (3).	0.154
53	Marginal scales / spicules—length relative to typical dorsal armature: absent or not differentiated (0); same length (1); shorter (2); substantially longer, three times length or longer (3).	0.200
54	Marginal scales / spicules—width relative to typical dorsal armature: absent or not differentiated (0); same width (1); narrower (2).	0.182
55	Marginal scales / spicules—texture: absent (0); smooth, strongly tapered (1); striated or grooved (2); smooth, cylindrical (3).	0.176
56	Marginal spicules—shape: absent (0); straight (1); distally curved (2).	0.222
57	Marginal spicules—terminal shape: absent (0); pointed, sharp (1); blunt (2).	0.154
58	Dorsal sutural spines differentiated from major armature: no (0); yes (1).	0.077
59	Marginal spicules—complex base: absent or simple (0); spicules in chitinous cupules (1); spicules in complex *rigschaftnadel* (2).	0.182
60	Ratio: animal body length / width: ≤2 (0); >2 (1).	0.063
61	Maximum adult body length (mm): <10 mm (0); ≥10 mm (1).	0.125
62	Radula—number of cusps on major lateral teeth: monocuspidate (0); bicuspidate (1); tricuspidate (2).	0.154
63	Radula—smallest cusp on major lateral teeth: monocuspidate or equal (0); anterior / interior (1); posterior / exterior (2); central, tricuspid with two outer denticles equal (3).	0.214
64	Gills—number per side in adult animal: ≤4 (0); 5-10 (1); 11-16 (2); >16 (3).	0.167
65	Gills—size distribution: largest at posterior, or all equal (0); smallest anterior and posterior (1).	0.100
66	Gills interrupted by anal interspace: yes (0); no, gills on anal stem (1).	0.125
67	Gills "merobranchial", posterior: yes (0); no, other arrangement (1).	1.000
68	Gill coverage, as percentage of foot length: <40% (0); ≥40% (1).	0.200
69	Gills abanal (single gill posterior of nephridiopore in adult animal): no (0); yes (1)	0.500

Table 2. Character-taxon data set utilized for the phylogenetic analysis. Asterisks (*) denote taxa designated as outgroups. Ingroup taxa are listed in alphabetical order by species epithet. For some characters, transitional states are coded r = {0,1}; t = {1,2}.

	1	2	3	4	5	6
	123456789012345678901234567890123456789012345678901234567890123456789
**Callochiton bouveti* Thiele, 1906	11000111101020110120101010100200001010033111 2??11101111220101223001 11
**Callochiton septemvalvis* (Montagu, 1803)	11000111100000110122101020100200001010022111 2?2111013212101012300111
**Choriplax grayi* (H. Adams and Angas, 1864)	11110001022022211121202011100110213 2?31??????????????????????1120?????1
Parachiton acuminiatus (Theile, 1909)	00010011001001000100102011110?r12122?32111112??2110231121001120???0?0
Leptochiton aequispinus (Bergenhayn, 1933)	01020000100020200110101020110200 21???2211211???000001131200010021 10?0
Leptochiton algesirensis (Capellini, 1859)	11010001010020?0110010?01?110?r01222112221 1t222121111212000110201010
Leptochiton alveolus (Sars MS, Lovén, 1846)	10011000000011?01?1010100??10100212 11?2112112?12110 2????1011002?10?0
Leptochiton americanus Kaas and Van Belle, 1985	11000001?10001201200101 11?10011021 1??3111111?1?21101?????0?0110110?0
Nierstraszella andamanica (Smith, 1906)	1110010010000r20110200002210120000120001111312?11202000010111021 1010
Leptochiton asellus (Gmelin, 1791)	110?0101101101?0??2220101?0101r02212122221122 1??????12111000101 1?1000
Leptochiton binghami (Boone, 1928)	???0?110???121????02101111?0210211?13131113 2?311010000000?01?0???0?0
Leptochiton bouchieti Sirenko, 2001	00010000000021001110101022110000001210031112122112223131112012 1000000
Lepidopleurus cajetanus (Poli, 1791) .	0001?01110?122?0??0100101211002011120?232111?122111t122110011202?1000
Leptochiton cancellatus (Jeffreys, 1839)	11010011111121001100101010110?10122211212112212211 1?????10100?111000
Parachiton communis Saito, 1996	01010011002000101100102010100001212 1?33111121?????000000011233 11000
Deshayesiella curvata (Capenter MS, Pilsbry, 1892)	01010111020120200?021??11000002021 2?1322111121?211011211 10?1110111000
Leptochiton deforgesi Sirenko, 2001	10010000000111001100102022110000112 1122312112122122111312121021000000
Leptochiton denhartogi Strack, 2003	11010011111121001100101011110010122 1112111111212211011121100000011 1000
Leptochiton foresti (Leloup, 1981)	11100100100010200102101012100210001 2000111 13?1?11202112210101201 1?010
Leptochiton hirasei (Taki and Taki, 1929)	00010001 10?12100020010 1?1?0000r0211??11121112 1?2110111211011020 11?0?0
Leptochiton inquinatus (Reeve, 1847)	01010011100000001100 10?01?1000r01211123121112 ?2212121121t1?11001?1000
Leptochiton intermedius (Salvini-Plawen, 1968)	10010001000001100100101012110000?????????????????????????????1?????0?0
Leptochiton japonicus (Theile, 1909)	01020000100020200110101020110200 21???2211211???000001131200010021 1000
Leptochiton juvenis (Leloup, 1981)	00000101000020101100111210110200211111212111212212001231111012321 1000
Leptochiton kerguelensis Haddon, 1886	00011011000011001?0000112?10000021 1?1211111112211 12????10001011?0?0
Nierstraszella lineata (Nierstrasz, 1905)	110r00010100012011110?203110113000120001111312?11202000010111021 1010

Table 2. (Continued)

	1	2	3	4	5	6
	123456789012345678901234567890123456789012345678901234567890123456789					
Leptochiton medinae (Plate, 1899)	000100010?0100001100101110100010112?1321121111222202112110001101110000					
Hanleya nagelfar (Lovén, 1846)	110010010000100000200000007000000020000121132??11101000000011203110100					
Leptochiton n. sp. 4 Sigwart, 2008a	000100010001200011201011111000002121122331112123122100000100021000000					
Leptochiton n. sp. 5 Sigwart, 2008a	000000000000210011001112221101002111221111111231221311121201210000000					
Oldroydia percrassa (Dall, 1894)	110001001100012012227?0111?10011000107?0013111212111017????00111021100?					
Leptochiton pergranatus Dall, 1889	??00000110?021100?021010111?02r0212?132331131223110100000?01?02??000					
Ferreiraella plana (Nierstrasz, 1905)	110201001021012012111010207?10100001200000000200112021121207?01203??0?0					
Parachiton politus Saito, 1996	010100100020001001001020101000012121?22211112 1?21202000000112331100 0					
Leptochiton rugatus (Capenter MS, Pilsbry, 1892)	??010011?1011?00110020?01?1?00r012??1t311211?1?22202112221??1001?1000					
Leptochiton saitoi Sirenko, 2001	001000010000201011001011111002000022000000032221211111111110023111000					
Leptochiton scabridus (Jeffreys, 1880)	000100011007?1100012011101?00011011 2?1131221 12?221111????1?00201?1000					
Leptochiton thandari Sirenko, 2001	100000001000000011001011110101002121121111112122121111111120021000000					
Leptochiton vanbellei Sirenko, 2001	100110000001100001001010221102000022000111212221122111311121121000000					
Leptochiton vaubani Kaas, 1991	110011000000110011101012211002202122031111112222110100000100111300010					
Leptochiton vietnamensis Sirenko, 1998	010001010000201001001010101000000022100000032221111111111110011211000					
Ferreiraella xylophaga Gowlett-Holmes and Jones, 1992	100000000200102112110000021102000020000000002001110111111111123301010					

50 permutation replicates (Faith and Cranston 1991). Having determined that the data differ significantly from random, they were then analyzed in the standard software package PAUP* version 4.0b10 (Swofford 2002), using a heuristic search algorithm. To improve the efficiency of analysis, an initial heuristic search was performed with ten random addition sequence replicates to determine the size (*n* steps) of the shortest common most parsimonious trees (MPTs). The analysis was then repeated with 100 random addition sequence replicates, limiting the search to trees of length *n* or less. All trees of minimum length were retained as the primary set of MPTs to a maximum of 3000 MPTs. To assess the relative degree of clade support, bootstrap values were calculated from the analysis, using 50 bootstrap replicates of 10 random addition sequence replicates each.

Strict consensus cladograms can show dramatic reduction of resolution when the positions of a few ingroups are highly mobile (*e.g.*, Wilkinson 1999). Reduced consensus methods can recover additional ingroup relationships by removing these unresolved taxa. It is important to note that taxa are pruned only after the analysis is performed using the complete data matrix so no data are selectively "ignored". This study employed the "Strict" program in the software package RedCon version 3.0 (Wilkinson 2001) to produce a set of strict reduced consensus trees.

Two methods were employed to examine the influence of the two major data partitions (shell characters and girdle characters). First, consensus index (CI) values for the two data sets were compared from within the main analysis (the value for each character of the minimum number of potential state changes, divided by the minimum number actually observed on the tree). Two secondary analyses were run on restricted sections of the matrix (one with shell characters only, one with girdle characters alone) with the same parameters and protocol as the main analysis (initial PTP test and heuristic search protocol in PAUP* outlined above).

RESULTS

The permutation tail probability calculated for the matrix was 0.02, which is significant ($P < 0.05$), indicating that the matrix should contain a resolvable phylogenetic signal. The phylogenetic analysis resulted in 68 MPTs, each of length 517

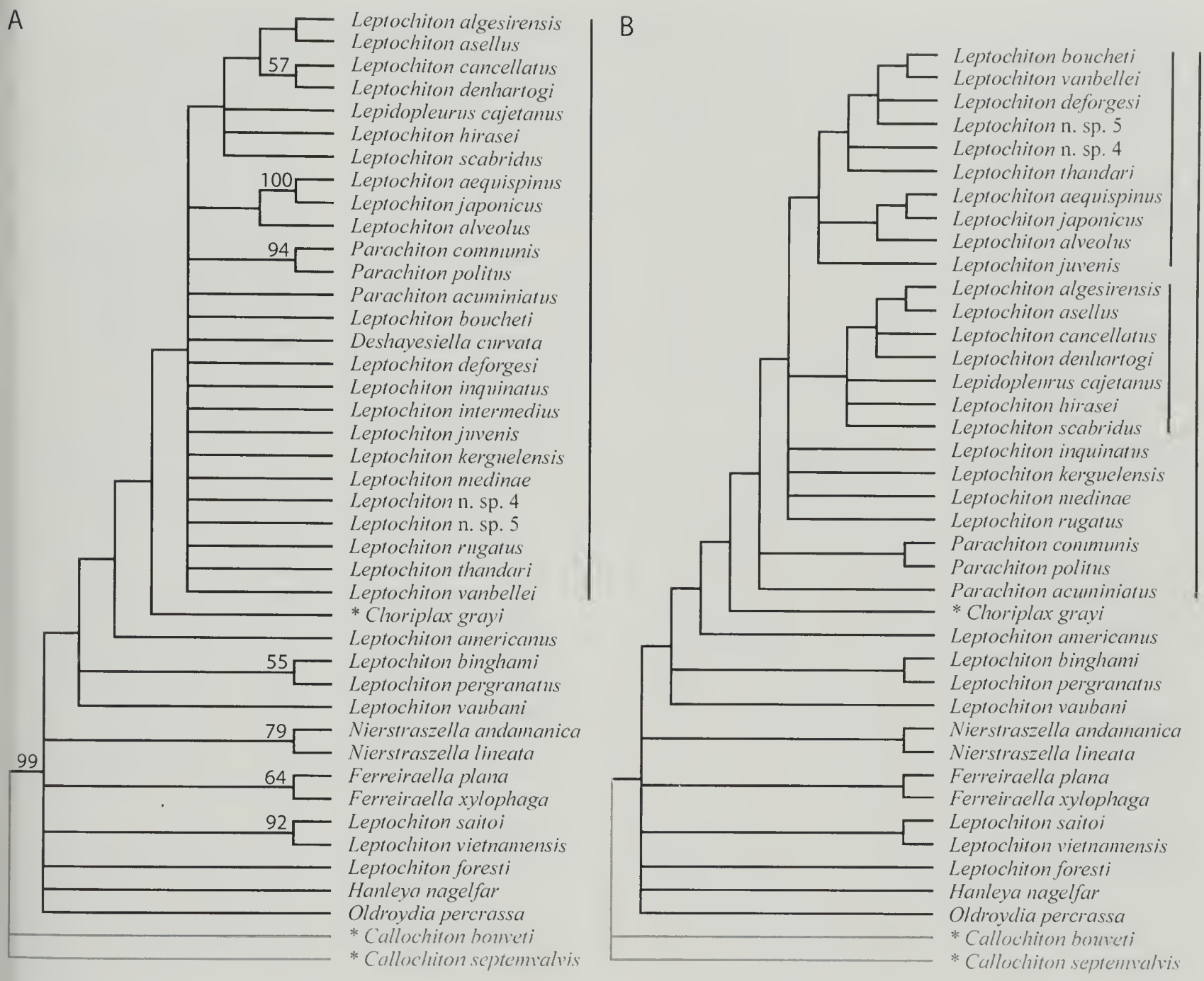

Figure 2. Phylogenetic trees resulting from analysis of morphological characters for Lepidopleurida. Asterisks (*) denote taxa designated as outgroups. A, strict consensus of 68 MPTs; bootstrap values are marked on internal nodes (where the bootstrap value is over 50). B, preferred tree, strict reduced consensus tree with *Leptochiton intermedius* and *Deshayesiella curvata* pruned from trees contributing to Fig. 1A. Major internal clades discussed in the text are marked with vertical lines to the right: predominantly Pacific *Leptochiton* spp. (top, *Leptochiton boucheti–Leptochiton juvenis*); predominantly Atlantic *Leptochiton* spp. (lower, *Leptochiton algesirensis–Leptochiton scabridus*); major *Leptochiton* clade including *Parachiton* (*Leptochiton boucheti–Parachiton acuminatus*).

steps (Fig. 2A). The strict consensus of these trees recovers a monophyletic Lepidopleurida including *Choriplax*, relative to the *Callochiton* outgroup (Fig. 2A). Whether *Choriplax* is designated an outgroup taxon or an ingroup taxon does not change the topology of the resulting trees. Un-rooted trees also resolve the same topology in all cases. Within the ingroup, a single major clade containing most *Leptochiton* species (21 of 28 taxa included in the analysis), including the type species *Leptochiton asellus* (Gmelin, 1791) as well as *Lepidopleurus* Risso, 1826, *Parachiton* Thiele, 1909, and *Deshayesiella* Carpenter in Dall, 1879. The remaining lepidopleurans are arranged as a grade sister to this main clade. Strong sister-taxa relationships resolve *Nierstraszella* Sirenko, 1992 and *Ferreiraella* Sirenko, 1988 as monophyletic (each genus represents its own family). The monophyly of Lepidopleurina is supported by three synapomorphies: merobranchial gill arrangement (unique to Lepidopleurina), adanal gill placement (although this is reversed in *Choriplax*), and lack of shell insertion plates (although this is reversed in *Ferreiraella* as well as in *Choriplax*).

Reduced consensus analysis resulted in a set of seven additional strict reduced consensus trees; four excluded a single taxon from the trees, two trees excluded two taxa, and one excluded nine taxa. Pruning *Leptochiton intermedius* (Salvini-Plawen, 1968) produced better resolution within the main *Leptochiton* clade. However, other permutations recovered in these seven SRC trees did not change the topology of the cladogram. The two SRC trees that pruned two taxa both excluded *L. intermedius* and one of *Parachiton acuminatus* (Theile, 1909) or *Deshayesiella curvata* (Capenter MS, Pilsbry, 1892). *Parachiton acuminatus* is the type species of the genus *Parachiton* so its exclusion is not preferred. The preferred final tree representing the phylogeny, based on this character matrix, is the SRC tree excluding *L. intermedius* and *D. curvata* (Fig. 2B).

Bootstrap values for the nodes recovered were generally low, with support (>50) for local sister-group relationships but low support (<50) for the major clade of *Leptochiton* and allies (Fig. 2A). Within this major *Leptochiton* clade, there is one consistently resolved group of primarily Atlantic species although one Japanese species, *Leptochiton hirasei* (Taki and Taki, 1929), is included. This is the only cluster of species that resolves consistently in all MPTs although bootstrap support for this clade is low. Reduced consensus methods further resolve a separate group of Pacific species of *Leptochiton* although one Atlantic species, *Leptochiton alveolus* (Sars MS, Lovén, 1846), is included (Fig. 2B).

Consistency index (CI) values for most characters were quite low (Table 1). The average CI values for the shell characters (0.22) were lower but not substantially different from the average CI for girdle characters (0.26). The analysis of 32 shell characters resulted in 348 MPTs of length 206 steps; analysis of 27 girdle characters resulted in a large number of MPTs (>3000), length 180 steps. Consensus trees from each of these analyses resulted in a totally unresolved polytomy that failed to separate the outgroup and ingroup taxa.

DISCUSSION

Lepidopleuran chitons are recognized by three major synapomorphies: the absence (or very minimal development) of shell insertion plates; latero-ventral shell eaves that anchor the valves in the fleshy girdle; and the posterior (*i.e.*, "merobranchial") and adanal gill arrangement. The adanal condition is identified by the placement of multiple gills posterior to the nephridiopore; gills are added to both the anterior and posterior ends of the gill row during ontogeny. These features (absent insertion plates and gill arrangement) are traditionally considered plesiomorphic within chitons. Fossil polyplacophoran valves with morphology very similar to those in living lepidopleurans are known in many taxa from the Lower Carboniferous (ca. 350 Mya), whereas shell insertion plates first appear in the fossil record in the Permian (ca. 290-250 Mya) and become more common in Cretaceous taxa and later. Merobranchial adanal gills, although not preserved in the fossil record, are a feature unique to lepidopleurans, and this is assumed to be the ancestral condition on that basis.

Sirenko (2006) revised the classification of Lepidopleurida and assigned the genera *Choriplax*, *Hemiarthrum*, and *Weedingia* to the family Hemiarthridae in the Chitonida. These three genera had previously been classified in the Lepidopleurida because, although they have shell insertion plates, the insertion plates are weak and unslitted. Their removal from the Lepidopleurida was based on their abanal gill arrangement (with only one gill located posterior to the nephridiopore in adult animals). Sirenko (2006) interpreted the gill arrangement in these three genera to be indicative that they are more derived and that the insertion plates in their shells are secondarily simplified. In the present analyis, *Choriplax* resolves within Lepidopleurina.

The nature of nephridiopore arrangement in lepidopleurans and all chitons clearly requires further study. Other genera that are uncontroversially considered to be within Chitonida may have adanal (plesiomorphic) gill arrangements; *Chaetopleura*, *Stenoplax*, *Callistochiton*, and *Onithochiton* species all have three or more gills posterior to the nephridiopore in adult animals. This classification of gill arrangements may not provide the straightforward synapomorphy that has been proposed.

In the present analysis, the large and dominant genus *Leptochiton* is clearly non-monophyletic. The major clade (Fig. 2B) could be considered to represent the family Leptochitonidae including *Parachiton*. Although the shell morphology that defines *Parachiton* (having a substantially enlarged tail valve) is distinctive, it may be interesting to consider in future studies whether that feature has evolved more than once. Outside of the major "Leptochitonidae" clade, the two other lepidopleuran families represented by more than one species are resolved in this tree: Nierstraszellidae (*Nierstraszella* spp.) and Ferreiraellidae (*Ferreiraella* spp.) as part of a basal polytomy including *Hanleya* (Hanleyidae) and *Oldroydia* (Protochitonidae). The consistent recovery of Mediterranean and North Atlantic taxa in close topological proximity (although not supported by bootstrap values) indicates that there may be a single regional radiation that links these taxa, which is worthy of further study. The two genera included, *Lepidopleurus* and *Leptochiton*, have been the subject of taxonomic controversy; the type species of both genera are included in this putative North Atlantic clade.

The first cladistic analysis published to assess the relationships within a clade of chitons was presented by Sigwart *et al.* (2007); as that analysis was intended to assess the phylogenetic

position of a new fossil species, it used only shell morphology. The resulting phylogeny did not recover any of the family or genus-level groups within Lepidopleurina although there were some relationships between individual sister taxa that were well supported (bootstrap >50), as was also found in this expanded study.

Understanding the potential usefulness of the major data sets available is an important step in building our understanding of the evolution of this group. The analysis of the two largest data partitions in this study, shell morphology characters and girdle morphology characters, unambiguously demonstrates that employing more characters produces more resolution. Analysis of either data set alone results in large numbers of trees with effectively random distribution of taxa. This was also demonstrated by the large number of initial MPTs recovered in the study of Sigwart *et al.* (2007) using shell character data.

In studies of molecular phylogenetic methods, it is well known that resolution and support recovered by phylogenetic analyses improves with increased character sampling (*i.e.*, number of genes and/or length of sequences). This argument suggests that adding more data of any type produces better resolved and presumably more accurate trees (*e.g.*, Poe and Swofford 1999, Simmons and Miya 2004). Questions remain unresolved about the relative benefits of adding more characters or more taxa in phylogenetic reconstruction, particularly using morphological data (Graybeal 1998, Cobbett *et al.* 2007). Some workers have even argued that large-scale cladistic data sets of morphological data are less informative than those concentrating on few well-described characters (Scotland *et al.* 2003). The basis for this position is that morphological data alone cannot provide a sufficient character base to derive robust phylogenetic hypotheses, so it may be more accurate to rely on molecular data and use a few broadly defined morphological features as a reassuring appendum. This has been informally called the "Christmas tree" approach to the use of morphological attributes: using morphological ornaments to make an adequate tree more aesthetic. In fact, one argument for the dominance of molecular phylogenetic methods to the exclusion of morphological data is that "much of the useful morphological diversity has already been scrutinized" (Scotland *et al.* 2003: 545). This statement is at odds with the state of anatomical knowledge for many biological groups, of which chitons are a quite typical example.

Other studies have concluded that "much more [morphological] variation could be included in phylogenetic analyses than is used presently" (Poe and Wiens 2000: 33-34). This is true not only of computational phylogenetic analyses but of the descriptive taxonomic studies that form a basis for phylogenetic investigation. The sources of data to describe species of chitons rely almost exclusively on external anatomy (gills, girdle, and shell morphology), with use of radular morphology (Saito 2004) and aesthete arrangement (Sirenko 2001, Fernandez *et al.* 2007) gaining popularity and regular usage. Further sources of data, such as gamete morphology (Buckland-Nicks 2006) and nephridiopore arrangement (Sirenko 1993, Sigwart 2008b) and others will become standard sources of morphological character data as anatomical descriptions are published for larger numbers of closely related taxa.

The present study, based on "classical" morphological characters used in traditional species descriptions of chitons, functions as a model to demonstrate that cladistic analyses of morphological data sets can successfully generate phylogenetic hypotheses. Whether or not the hypotheses proposed by these cladograms are "true" will be determined by the progressive accumulation of a body of work examining the internal relationships of taxa within well-established clades, based on multiple data sets including genetic and novel morphological information.

Morphological data that are available from currently routine description and analysis of specimens can be used to produce phylogenetic hypotheses and provide new insights into the relationships within known clades. The anecdotal superficial homogeneity of chiton morphology is disputed by this finding. Maximizing the number of morphological characters in analysis improves the quality of phylogenetic signal recovered (making the transition from a complete polytomy to resolved clades). Application of morphological data is critical not only because it is sensible to use all available data for living taxa, but also because it creates a link to fossil taxa. Encompassing all forms of data for fossil and living species is critical to understanding the ongoing evolution of chitons and other groups of living fossils.

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